

Applicant : Andrew W. Shy
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Attorney's Docket No.: 07334-004009

REMARKS

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be examined. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

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Version with markings to show changes made

In the specification:

Paragraph beginning at page 1, line 5, has been amended as follows.

[This is a continuation-in-part of U.S. Serial No. 08/412,431.] This application is a continuation of application serial number 09/454,854, filed December 7, 1999, which is a divisional of application serial number 09/181,030, filed October 27, 1998, which is a continuation of application serial number 08/862,442, filed May 23, 1997, which is a divisional of application serial number 08/623,679, filed March 29, 1996, now U.S. Patent No. 5,674,739, which is a continuation-in-part of application serial number 08/412,431, filed March 29, 1995, now U.S. Patent No. 5,633,161.

Paragraph beginning at page 15, line 26, has been amended as follows:

[FIGS. 3A and 3B] FIGS. 3A-3C are representations of the nucleotide and derived amino acid sequences of cDNA clone fomy030 (SEQ ID NOs:2 [nucleotide sequence] and 3 [amino acid sequence]) derived from fomy030 mRNA.

Paragraph beginning at page 16, line 3, has been amended as follows:

[FIG. 5] FIGS. 5A-5G is a representation of the nucleotide and deduced amino acid sequences of cDNA clone of foxy030 (SEQ ID NOs:6 [nucleotide sequence] and 7 [amino acid sequence]).

Paragraph beginning at page 16, line 6, has been amended as follows:

[FIG. 6] FIGS. 6A-6H is a comparison of the nucleotide and deduced amino acid sequences of another cDNA clone of foxy030 (SEQ ID NOs:8 [nucleotide sequence] and 9 [amino acid sequence]).

Paragraph beginning at page 41, line 2, has been amended as follows:

The differentially expressed and pathway genes of the invention are listed below, in Table 1. The nucleotide sequence for the differentially expressed fomy030 gene is shown in FIGS. 2 and [3A and 3B] 3A and 3C. Specifically, FIG. 2 depicts the nucleotide sequence (SEQ ID NO:1) of the amplified cDNA band initially identified via differential display analysis, which is referred to herein as romy030. FIGS. [3A AND 3B] 3A and 3C depict the nucleotide

sequence (SEQ ID NO:2) of a fomy030 cDNA clone which was isolated using a romy030 probe. The deduced amino acid sequence also is shown in FIGS. [3A and 3B] 3A and 3C (SEQ ID NO:3). [FIG. 5] FIGS. 5A-5G shows the nucleotide (SEQ ID NO:6) and deduced amino acid sequences (SEQ ID NO:7) of a foxy030 cDNA clone which was isolated using the entire mouse fomy030 cDNA as a probe. [FIG. 6] FIGS. 6A-6H shows an alternative splice form of foxy030 (SEQ ID NOS:8 and 9).

Paragraph beginning at page 42, line 25, has been amended as follows:

Finally, nucleotide sequences contained within the differentially expressed genes are listed in the Figures indicated under the heading "Seq." In the case of the fomy030 gene, such sequences are listed in FIGS. 2 and [3A and 3B] 3A and 3C, and for foxy030, in FIGS. [5 and 6] 5A-5G and 6A-6H.

Paragraph beginning at page 43, line 7, has been amended as follows:

The genes listed in Table 1 can be obtained using cloning methods well known to those skilled in the art, including, but not limited to, the use of appropriate probes to detect the genes within an appropriate cDNA or gDNA (genomic DNA) library. (See, for example, Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, which is incorporated by reference herein in its entirety). Probes for the novel sequences reported herein can be obtained directly from the isolated clones deposited with the NRRL, as indicated in Table 2, below. Alternatively, oligonucleotide probes for the novel genes can be synthesized, using techniques well known to those of skill in the art, based on the DNA sequences disclosed herein in FIGS. 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and 6A-6H.

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The Table on page 43 has been amended as follows:

TABLE 1
Differentially Expressed and Pathway Genes

| GENE | Sequence ID | Paradigm of Original Detection (↑/↓) | Paradigm Expression Pattern | Cell Type Detected in | Ref. | Seq. |
|---------|-------------|--------------------------------------|-----------------------------|-----------------------|------|---|
| fomy030 | 2 | B16 ↑ F1 B16 ↓ F10 | melanoma cells | melanocyte | | Fig. 2, [3A & 3B |
| fohy030 | 6 & 8 | benign nevi ↑ malignant melanoma ↓ | biopsy samples | melanocyte | | Fig. 5 & 6] <u>3A-3C, 5A-5G & 6A-6H</u> |

Paragraph beginning at page 44, line 15, has been amended as follows:

As used herein, "differentially expressed gene" (i.e., target and fingerprint genes) or "pathway gene" refers to (a) a gene containing: at least one of the DNA sequences disclosed herein (as shown in FIGS. 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and 6A-6H) or contained in the clones listed in Table 2, as deposited with the NRRL; (b) any DNA sequence that encodes the amino acid sequence encoded by: the DNA sequences disclosed herein (as shown in FIGS. 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and 6A-6H), contained in the clones, listed in Table 2, as deposited with the NRRL or contained within the coding region of the gene to which the DNA sequences disclosed herein (as shown in FIGS. 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and 6A-6H) or contained in the clones listed in Table 2, as deposited with the NRRL, belong; (c) any DNA sequence that hybridizes to the complement of: the coding sequences disclosed herein (as shown in FIGS 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and 6A-6H), contained in clones listed in Table 2, as deposited with the NRRL, or contained within the coding region of the gene to which the DNA sequences disclosed herein (as shown in FIGS. 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and

6A-6H) or contained in the clones listed in Table 2, as deposited with the NRRL, belong under highly stringent conditions, *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. *et al.*, 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a gene product functionally equivalent to a gene product encoded by a gene of (a), above and/or (d) any DNA sequence that hybridizes to the complement of: the coding sequences disclosed herein, (as shown in FIGS. 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and 6A-6H) contained in the clones listed in Table 2, as deposited with the NRRL or contained within the coding region of the gene to which DNA sequences disclosed herein (as shown in FIGS. 2, [3A, 3B, 5, and 6] 3A-3C, 5A-5G, and 6A-6H) or contained in the clones, listed in Table 2, as deposited with the NRRL, belong under less stringent conditions, such as moderately stringent conditions, *e.g.*, washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel *et al.*, 1989, *supra*), yet which still encodes a gene product functionally equivalent to a gene product encoded by a gene of (a), above.

Paragraph beginning at page 119, line 29, has been amended as follows:

Three independent library isolated cDNAs, as well as a cDNA isolated as a 3' RACE product were found to contain the fomy030 sequence. Thus, the most probable explanation for the divergence of the human and murine sequences is the existence of alternate splice forms of the fomy030 and foxy030 transcripts. The fomy030 splice version results in a protein product of 542 amino acids in length, while the foxy030 splice variant is predicted to encode a protein of 1497 amino acids in length ([FIG. 5] FIGS. 5A-5G).

Paragraphs beginning at page 120, line 6, has been amended as follows:

Another splice variant is shown in [FIG. 6] FIGS. 6A-6H (SEQ ID NO:8), and encodes a protein of 1533 amino acids in length (SEQ ID NO:9). The cDNA of [FIG. 5] FIGS. 5A-5G (SEQ ID NO:6) is missing 34 nucleotides beginning after 2879 in SEQ ID NO:8, and is missing 74 nucleotides beginning after 2926 in SEQ ID NO:8. Thus, nucleotides 2880-2892 in SEQ ID NO:6 are identical to nucleotides 2914-2926 in SEQ ID NO:8, and the sequences are essentially identical starting at 2893 in SEQ ID NO:6 and 3001 in SEQ ID NO:8. The difference in the respective amino acid sequences is that the amino acids are identical from 1 to 844, and then again from 850 to 1497 in SEQ ID NO:7 and from 886 to 1533 in SEQ ID NO:9.

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Within their common 5' sequences, fohy030 was also found to have an additional three base pairs (GGA) inserted after position 1394 in the mouse cDNA (at positions 1066-1068 in FIGS. [5 and 6] 5A-5G and 6A-6H). These additional three base pairs fall within the open reading frames of both fohy030 and fomy030, and result in an additional Glycine residue at position 356 within the open reading frame of fohy030 relative to fomy030.

Paragraph beginning at page 124, line18, has been amended as follows:

Two specific oligonucleotides were generated based on the sequence of romy030, romy030U 5'-GGGGAAAGCACATCAAGGAAC-3' (SEQ ID NO:4) and romy030L 5'-GCAACTACACTCGGAAAAGC-3' (SEQ ID NO:5), for use in PCR reactions. cDNA libraries prepared from mRNA isolated from normal melanocytes and a mouse melanoma cell line were analyzed for the presence of fomy030 by PCR, utilizing the above romy030 probes. Fomy030 was detected in the melanocyte library but not in the melanoma library. The melanoma library was generated from a highly metastatic mouse melanoma K-1735 m2. This result is consistent with the observation that fomy030 is present at reduced levels in the metastatic B16 F10 melanoma cell line. A radioactive DNA probe was generated from the subcloned romy030 DNA. This probe was used to screen the normal mouse melanocyte cDNA library. Three independent positive clones were identified and isolated during this screening. These clones were designated fomy030a, fomy030b, and fomy030c. These cDNAs were sequenced and the overlapping portions were found to be identical. The nucleotide sequence of all three fomy030 cDNAs, designated as the fomy030 sequence (SEQ ID NO:2) is depicted in FIGS. [3A and 3B] 3A-3C, and contains the sequence of romy030. The findings described herein suggest a novel role for fomy030 in tumor progression. A down-regulation of 030 can be used as a diagnostic marker for tumor progression, especially for the progression to metastasis. Further, 030 gene products can be used in the prevention and treatment of tumor progression disorders.

Pages 130 - 161 have been deleted.